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(54) Title: METHOD OF TREATING BLADDER AND LOWER URINARY TRACT SYNDROMES

(57) Abstract

The present invention relates to bladder and lower urinary tract syndromes, particularly, irritative symptoms, and to a method of treating same using  $\alpha_{1d}$ -adrenergic receptor ( $\alpha_{1d}$ AR) antagonists. The invention further relates to a method of screening compounds for their ability to serve as  $\alpha_{1d}$ AR selective antagonists.

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Exhibit 2

## METHOD OF TREATING BLADDER AND LOWER URINARY TRACT SYNDROMES

This application claims priority from US Provisional Application Number 5 60/084,479, filed May 6, 1998, the entire contents of that application being incorporated herein by reference.

### TECHNICAL FIELD

The present invention relates to bladder and lower urinary tract syndromes, particularly, irritative symptoms, and to a method of treating same 10 using  $\alpha_{1d}$ -adrenergic receptor ( $\alpha_{1d}$ AR) antagonists. The invention further relates to a method of screening compounds for their ability to serve as  $\alpha_{1d}$ AR antagonists.

### BACKGROUND

Lower urinary tract symptoms (LUTS) resulting from bladder outlet obstruction (BOO) remains one of the most commonly encountered disorders in 15 urology, and can be secondary to fixed anatomical and/or functional causes (Steers et al, Voiding dysfunction: diagnosis, classification, and management, in Adult and Pediatric Urology; Third Edition, J.Y. Gillenwater, et al., Editors, 1996, Mosby-Year Book, Inc.: St. Louis. p. 1220-1325.). Causes of BOO include 20 prostatic enlargement (benign or malignant), bladder neck contracture, urethral stricture, and meatal stricture (Steers et al. Voiding dysfunction: diagnosis, classification, and management, in Adult and Pediatric Urology: Third Edition, J.Y. Gillenwater, et al., Editors. 1996, Mosby-Year Book, Inc.: St. Louis. p. 1220-1325.). Symptoms associated with BOO typically fall into obstructive or irritative 25 categories; obstructive symptoms include hesitancy, poor stream, prolonged urination, and feelings of incomplete emptying, while irritative symptoms consist

of frequency, urgency, nocturia, and unstable bladder contractions. The bladder is functionally and anatomically divided into the detrusor (body and ventral base) and trigone (dorsal portion of base extending between the ureteral orifices and the bladder neck) (Zderic et al, Voiding function: relevant anatomy, physiology, 5 pharmacology, and molecular aspects, in *Adult and Pediatric Urology*; Third Edition, J.Y. Gillenwater, et al., Editors. 1996, Mosby-Year Book, Inc.: St. Louis. p. 1159-1219), with distinct histology, histochemistry, and pharmacology. In contrast, the prostate and trigone have similar vascular supply, innervation, and receptor expression (Gosling et al, Detrusor morphology in relation to bladder 10 outflow obstruction and instability, in *Benign Prostatic Hypertrophy*, F. Hinman, Editor. 1983, Springer-Verlag: Berlin. p. 666-71).

The physiology of LUTS secondary to benign prostatic hypertrophy (BPH) has two components: (1) a static component related to the increase in 15 prostatic cellular mass and (2) a dynamic component related to variations in prostatic smooth muscle tone (Caine et al, *Brit. J. Urol.* 47:193-202 (1975)). Histologically BPH is characterized by glandular (epithelial) and stromal (fibromuscular) hyperplasia, with the latter being the dominant factor in the pathogenesis of clinically significant BPH (Shapiro et al, *J. Urol.* 147:1293-1297 20 (1992)). Therefore much attention has focused on the role of the sympathetic nervous system and  $\alpha_1$ -adrenergic receptors ( $\alpha_1$ ARs) in the dynamic component of BOO, leading to clinical studies of  $\alpha_1$ AR antagonists as agents to relieve outlet obstruction. These studies have found that  $\alpha_1$ AR antagonists relax prostatic smooth muscle, relieving obstructive symptoms (Chapple, *Brit. J. Urol.* 1:47-55 25 (1995), Caine, *Urol. Clin. N. Am.* 17:641-649 (1990), Kawabe and Niijima, *Urol. Int.* 42:280-284 (1987), Lepor et al, *J. Urol.* 148:1467-1474 (1992), Reuther and

Aagaard, Urol. Int. 39:312-313 (1984), Matyus and Horvath, Med. Res. Rev. 17:523-535 (1997)). In addition,  $\alpha_1$ AR antagonists have been found to relieve the irritative bladder symptoms in men (most often associated with BPH) and women (Matyus and Horvath, Med. Res. Rev. 17:523-535 (1997), Serels and Stein, 5 Neurol. Urodyn. 17:31-36 (1998)). While it is logical to assume that elimination of BOO would relieve irritative symptoms, a number of recent studies suggest that the relationship between bladder irritability and outlet obstruction is not straightforward (Caine, Urol. Clin. N. Am. 17:641-649 (1990), Chapple and Smith, Brit. J. Urol. 73:117-123 (1994), Steers and De, J. Urol. 140:864-71 10 (1988), Steers et al, Am. J. Physiol. 266:R20 (1994)).

$\alpha_1$ ARs are members of the larger family of G protein-coupled adrenergic receptors which mediate actions of the endogenous catecholamines norepinephrine (NE) and epinephrine, resulting in smooth muscle contraction. cDNAs encoding three distinct  $\alpha_1$ AR subtypes ( $\alpha_{1a}$ ,  $\alpha_{1b}$ ,  $\alpha_{1d}$ ) have been cloned, 15 expressed in cells, and resultant protein characterized pharmacologically (Schwinn et al, J. Pharmacol. Exper. Ther. 272:134-142 (1995), Hieble et al, Pharmacol. Rev. 47:267-70 (1995)).  $\alpha_{1a}$ ARs predominate in prostate and bladder trigone (Price et al, J. Urol. 150:546-551 (1993)), and have been shown to be functionally important in mediating prostate smooth muscle contraction (Forray et 20 al, Mol. Pharmacol. 45:703-708 (1994), Lepor et al., J. Pharmacol. Exper. Ther. 270:722-727 (1994)). In addition to the three cloned  $\alpha_1$ AR subtypes which have high affinity for the antagonist prazosin, a fourth type of  $\alpha_1$ AR with low affinity for prazosin ( $\alpha_{1L}$ ) has been postulated (Muramatsu et al, Brit. J. Urol. 74:572-578 25 (1994)). In spite of initial evidence suggesting a role for the  $\alpha_{1L}$ AR in human prostate smooth muscle contraction (Ford et al, Mol. Pharmacol. 49:209-215 (1996)), more recent data suggests RS17053 (the compound used in these studies)

detects a low affinity state of the  $\alpha_{1a}$ AR in tissues rather than a distinct  $\alpha_{1L}$ AR (Ford et al, Br. J. Pharmacol. 121:1127-1135 (1997)). Since non-selective  $\alpha_1$ AR antagonists currently used to treat BPH have undesirable side-effects including light headedness, dizziness, and asthenia (Carruthers, Drug Safety 11:12-20 (1994)), many investigators have suggested that  $\alpha_{1a}$ AR subtype selective antagonists might be beneficial in improving BPH-related symptoms via relieving BOO (Matyus and Horvath, Med. Res. Rev. 17:523-535 (1997), Hieble and Ruffolo, Jr., Exp. Opin. Invest. Drugs 6:367-387 (1997)). However, this approach does not take into account that irritative symptoms may persist in spite of relief of outlet obstruction (Hieble and Ruffolo, Jr., Exp. Opin. Invest. Drugs 6:367-387 (1997)).

Very little information exists regarding the role of  $\alpha_1$ ARs in human detrusor. One of the few studies addressing this issue suggests human bladder (dome) contains only  $\alpha_{1a}$ ARs (Walden et al, J. Urol. 157:1032-1038 (1997)). However, since irritative bladder symptoms persist in some patients despite relief of BOO, nonselective  $\alpha_1$ AR antagonists may relieve the irritative effects of BPH through direct effects on bladder detrusor or other sites involved in micturition. The present invention results from the realization that human detrusor expresses two  $\alpha_1$ AR subtypes ( $\alpha_{1d} > \alpha_{1a}$ ). This realization makes possible the identification of  $\alpha_1$ AR subtype selective antagonists that can be used to treat irritative symptoms.

#### SUMMARY OF THE INVENTION

The present invention relates generally to bladder and lower urinary tract syndromes and, more particularly, to a method of identifying  $\alpha_{1d}$ AR antagonists that can be used to treat irritative symptoms. The invention also relates to a method of treating irritative symptoms using such agents.

Objects and advantages of the invention will be apparent from the detailed description that follows.

#### BRIEF DESCRIPTION OF THE DRAWINGS

##### **FIGURES 1A-1C.** Schematic of the location of $\alpha_1$ AR subtype probes.

5      Highlighted in bold are regions of  $\alpha_{1a}$  (Fig. 1A),  $\alpha_{1b}$  (Fig. 1B), and  $\alpha_{1d}$  (Fig. 1C) ARs encoded by probes used in RNase protection assays.

10     **FIGURE 2.** Representative saturation binding isotherm generated using increasing concentrations of the  $\alpha_1$ AR radiolabeled antagonist [ $^{125}$ I]HEAT in human detrusor membranes.  $K_d$  is  $130 \pm 1.09$  pM ( $n=5$ ), similar to that reported for cells stably expressing each cloned human  $\alpha_1$ AR subtype (Schwinn et al, J Pharmacol. Exper. Ther. 272:134-142 (1995) -  $\alpha_{1a/d}$ AR of the reference refers to the  $\alpha_{1d}$ AR subtype described herein since the  $\alpha_1$ AR nomenclature used here is the IUPHAR nomenclature (Hieble et al, Phar. Rev. 97:267 (1995)).

15     **FIGURE 3.** RNase protection assays examining  $\alpha_1$ AR subtype expression in detrusor were performed in all patients ( $n=13$ ). A representative RNase protection assay showing results from five patients is shown. In this experiment, radiolabeled probe for each  $\alpha_1$ AR subtype is shown at the far left along with (from left to right) protected fragments resulting from total RNA extracted from rat-1 fibroblast cells stably expressing each cloned human  $\alpha_1$ AR subtype (20 mg; positive probe control), yeast tRNA (20 mg; negative control); and total RNA isolated from human detrusor (20 mg) from five patients (lanes 1-20). Gel exposure times are 24 hrs for probe and positive control lanes and 72 hrs for tRNA and human detrusor samples. Although the  $\alpha_{1d}$ AR subtype mRNA band is stronger than the  $\alpha_{1a}$ AR protected fragment, the  $\alpha_{1a}$ AR probe contains 73%

more radiolabeled  $\alpha$ UTP compared with the  $\alpha_{1d}$ ; hence, after normalization for radioactive label incorporation, two-fold predominance of the  $\alpha_{1d}$ AR subtype in human detrusor is apparent.

**FIGURES 4A and 4B.** Results from RT-PCR experiments on human  
5 detrusor (Fig. 4A) and rat whole bladder (Fig. 4B) RNA.  $\alpha_1$ AR subtype specific  
cDNA in plasmid vectors served as positive controls.

**FIGURE 5.**  $\alpha_1$ AR subtype expression in human detrusor was determined  
using competition analysis with the  $\alpha_{1d}$ AR-subtype selective ligand BMY7378.  
Results from a representative curve are shown demonstrating a two-site fit with  
10 high affinity  $K_i$  corresponding to the  $\alpha_{1d}$ AR.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the recognition of  $\alpha_{1d}$ AR as the  $\alpha_1$ AR subtype responsible for irritative symptoms associated with bladder and lower urinary tract diseases. The invention provides, in one embodiment, a method of  
15 selecting  $\alpha_{1d}$ AR antagonists and, in a further embodiment, a method of treating irritative symptoms using  $\alpha_{1d}$ AR antagonists. (The nomenclature used herein is the new nomenclature provided in Hieble et al, Phar. Rev. 97:267 (1995)).

The method of treatment to which the invention relates comprises  
administering to a patient suffering irritative symptoms an amount of an  $\alpha_{1d}$ AR  
20 antagonist sufficient to relieve such symptoms. In accordance with the invention, irritative symptoms include excessive frequency of urination, urgency of urination, nocturia and unstable bladder contractions. Patients amenable to treatment include men and women, children and adults. In males, a preferred antagonist is both an  $\alpha_{1a}$ AR and an  $\alpha_{1d}$ AR antagonist. In females, preferred

antagonists are  $\alpha_{1d}$ AR specific antagonists. The amount of the antagonist to be administered and the treatment regimen will vary with the antagonist, the patient and the effect sought. Optimum doses and regimens, however, can be readily determined by one skilled in the relevant art.

5        The present invention also relates to a method of screening compounds for their ability to bind primarily to  $\alpha_{1d}$ AR and thereby to function, potentially, as  $\alpha_{1d}$ AR antagonists. Preferred  $\alpha_{1d}$ AR selective antagonists show at least a two fold selectivity for  $\alpha_{1d}$ AR relative to  $\alpha_{1a}$ AR or  $\alpha_{1b}$ AR. Binding assays of this embodiment invention include cell-free assays in which  $\alpha_{1d}$ AR, or portion thereof (e.g. relevant 10 transmembrane portion -- see, generally, Hwa et al, *J. Biol. Chem.* 271:7956 (1996)), is incubated with a test compound (proteinaceous or non-proteinaceous) which, advantageously, bears a detectable label (e.g., a radioactive or fluorescent label). Preparations of membranes that bear  $\alpha_{1d}$ AR can be used in this assay, including commercially available preparations (e.g. the NEN multireceptor kit (NET 1034)). 15        Following incubation, the  $\alpha_{1d}$ AR, or portion thereof, free or bound to test compound, can be separated from unbound test compound using any of a variety of techniques (for example, the  $\alpha_{1d}$ AR (or portion thereof) (e.g., associated with a membrane) can be bound to a solid support (e.g., a plate or a column) and washed free of unbound test compound). The amount of test compound bound to  $\alpha_{1d}$ AR, or portion thereof, is 20 then determined using a technique appropriate for detecting the label used (e.g., liquid scintillation counting in the case of a radiolabelled test compound). (See Schwinn et al, *J. Pharm. Exp. Ther.* 272:134 (1995).)

25        Binding assays of this embodiment can also take the form of cell-free competition binding assays. Such assays can be conducted as described in the Examples that follow (see particularly Example 2 (the test compound being

substituted for BMY 7378 ) - see also Schwinn et al, J. Pharm. Exp. Ther. 272:134 (1995)). Alternatively,  $\alpha_{1d}$ AR, or portion thereof, can be incubated with a compound known to interact, specifically, with  $\alpha_{1d}$ AR (e.g., BMY7378), which compound, advantageously, bears a detectable label (e.g., a radioactive or fluorescent label). A 5 test compound (proteinaceous or non-proteinaceous) is added to the reaction and assayed for its ability to compete with the known (labeled) compound for binding to  $\alpha_{1d}$ AR, or portion thereof. Free known (labeled) compound can be separated from bound known compound, and the amount of bound known compound determined to assess the ability of the test compound to compete. This assay can be formatted so as 10 to facilitate screening of large numbers of test compounds by linking the  $\alpha_{1d}$ AR, or portion thereof or, to a solid support so that it can be readily washed free of unbound reactants.

$\alpha_{1d}$ AR, or portion thereof, suitable for use in the cell-free assays described above can be isolated from natural sources (e.g., as membrane preparations derived 15 from bladder, e.g., human bladder) or prepared recombinantly or chemically. The  $\alpha_{1d}$ AR, or portion thereof, or can be prepared as a fusion protein using, for example, known recombinant techniques. Preferred fusion proteins include a HIS tag, a FLAG tag, a GFP tag or other tag (moiety) suitable for use in colorimetric assays. Typically, the non- $\alpha_{1d}$ AR moiety is present in the fusion protein N-terminal to the  $\alpha_{1d}$ AR, or 20 portion thereof domain, but it can also be C-terminal.

As indicated above, the  $\alpha_{1d}$ AR, or portion thereof, can be present linked to a solid support, including a plastic or glass plate or bead, a chromatographic resin, a filter or a membrane. Methods of attachment of proteins, or membranes containing same, to such supports are well known in the art.

The binding assays of the invention also include cell-based assays in which  $\alpha_{1d}$ AR, or portion thereof, is associated with the cell membrane of an intact cell. Cells suitable for use in such assays include cells that naturally express  $\alpha_{1d}$ AR and cells that have been engineered to express, advantageously, over express,  $\alpha_{1d}$ AR (or portion thereof). Advantageously, cells expressing human  $\alpha_{1d}$ AR are used. Suitable cells are preferably eucaryotic, including mammalian (human and nonhuman) cells, insect cells and yeast cells.

Cells can be engineered to express  $\alpha_{1d}$ AR (advantageously, human  $\alpha_{1d}$ AR, or portion thereof) by introducing into a selected host (e.g. a eucaryotic host) an expression construct comprising a sequence encoding  $\alpha_{1d}$ AR, or portion thereof, operably linked to a promoter. A variety of vectors and promoters can be used. (See Schwinn et al, J. Pharm. Exp. Ther. 272:134 (1995).) Introduction of the construct into the host can be effected using any of a variety of standard transfection/ transformation protocols (see Molecular Biology, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor Press, 1989). Cells thus produced can be cultured using established culture techniques suitable for the involved host. Culture conditions can be optimized to ensure expression of the  $\alpha_{1d}$ AR (or portion thereof) encoding sequence.

While for the cell-based binding assays it is appropriate that the  $\alpha_{1d}$ AR (or portion thereof) be associated with the cell membrane, for other purposes the expression product can be secreted into the culture medium or present in the cell cytoplasm.

The cell-based binding assays of the invention can be carried out essentially as described above with respect to the cell free assays. Advantageously, the cell used

expresses predominantly the  $\alpha_{1d}$ AR subtype. By way of example, the cell-based binding assay can be carried out by adding test compound (advantageously, bearing a detectable (e.g., radioactive or fluorescent) label), to medium in which the  $\alpha_{1d}$ AR (or portion thereof) expressing cells are cultured, incubating the test compound with the 5 cells under conditions favorable to binding and then removing unbound test compound and determining the amount of test compound associated with the cells.

As in the case of the cell-free assays, the cell-based assays can also take the form of competitive assays, as described above. For example, a compound known to bind  $\alpha_{1d}$ AR (and preferably labelled with a detectable label) can be incubated with 10  $\alpha_{1d}$ AR (or portion thereof) expressing cells in the presence and absence of test compound. The affinity of a test compound for  $\alpha_{1d}$ AR can be assessed by determining the amount of known compound associated with the cells incubated in the presence of the test compound, as compared to the amount associated with the cells in the absence of the test compound.

15 A test compound identified in one or more of the above-described assays as being capable of binding to  $\alpha_{1d}$ AR can, potentially, serve as an  $\alpha_{1d}$ AR antagonist and therefore be suitable for use in the irritative symptom treatment method of the invention. To determine the specific effect of any particular test compound selected 20 on the basis of its ability to bind  $\alpha_{1d}$ AR, various assays can be used including IP assays (see Schwinn et al, J. Pharm. Exp. Ther. 272:134 (1995)) and bladder (e.g. human bladder) smooth muscle contraction assays (Ford et al, Mol. Pharm. 49:209 (1996)). Compounds suitable for use in treating irritative symptoms will be associated with antagonistic (inhibitory) effects in the IP assay and contraction inhibitory effects in the contraction assay.

In another embodiment, the invention relates to compounds identified using the above-described assays as being  $\alpha_{1d}$ AR antagonist. The compounds identified in accordance with the above assays can be formulated as pharmaceutical compositions. Such compositions comprise the compound and a pharmaceutically acceptable diluent or carrier. The compound can be present in dosage unit form (e.g., as a tablet or capsule) or as a solution, preferably sterile, particularly when administration by injection is anticipated. The dose and dosage regimen will vary, for example, with the patient, the compound and the effect sought. Optimum doses and regimens can be determined readily by one skilled in the art.

10 In another embodiment, the invention relates to antibodies specific for  $\alpha_{1d}$ AR, and antigen binding fragments thereof, including F(ab)<sub>2</sub>' or F(ab) fragments. The antibodies can be monoclonal or polyclonal and can be prepared using standard techniques. The antibodies can be used in  $\alpha_{1d}$ AR purification protocols or the antibodies can be formulated as pharmaceutical compositions and used

15 therapeutically as  $\alpha_{1d}$ AR antagonists.

In yet another embodiment, the present invention relates to a gene therapy approach to treating irritative symptoms. In this embodiment, oligonucleotides (constructs) are used that, upon administration, result in the production of a molecule that down regulates production of  $\alpha_{1d}$ AR. In a related embodiment, the present

20 invention relates to  $\alpha_{1d}$ AR antisense constructs and to a method of using same to treat irritative symptoms. Such constructs can be designed to target any of a variety of regions of the  $\alpha_{1d}$ AR gene, including the encoding sequence (e.g., regions encoding the intracellular portion that interacts with G protein and participates in the signal transduction pathway) and the 5'-untranslated region.

Delivery of the above-described constructs can be effected using any of a variety of approaches, including installation into the bladder (e.g. via the uretha) and introduction into the cerebrospinal fluid. The constructs can also be administered systemically, in which case targeting can be effected using, for example, smooth 5 muscle (e.g. bladder smooth muscle) specific promoters.

Effective vectors for use in the above-described gene therapy/antisense embodiments include viral vectors, such as retroviral vectors, adenoviral vectors and adenoassociated viral vectors. The constructs can also be present in association with a lipid, e.g. a liposome. (For details of antisense constructs and delivery systems, etc. 10 see, for example, Wagner *Nature* 372:333 (1994).) The amount of construct to be administered will vary, for example, with the construct, the patient and the effect sought. One skilled in the relevant art can readily optimize the dose and treatment regimen.

In yet another embodiment, the invention relates to kits, for example, kits 15 suitable for conducting assays described herein. Such kits can include  $\alpha_{1d}$ AR, or portion thereof, for example, bound to a solid support. The kit can include an  $\alpha_{1d}$ AR-encoding sequence,  $\alpha_{1d}$ AR antisense construct or  $\alpha_{1d}$ AR-specific antibody. The kit can include any of the above components disposed within one or more container means. The kit can further include ancillary reagents (e.g., buffers) for use in the 20 assays.

Certain aspects of the present invention are described in greater detail in the non-limiting Examples that follow.

EXAMPLES

The following experimental details are relevant to the specific Examples that follow.

5       **Tissue preparation.** Full-thickness human bladder detrusor was obtained as discarded "normal" tissue adjacent to tumor specimens (n=1 radical cystectomy, n=12 radical cystoprostatectomy for transitional cell carcinoma of the bladder) with appropriate institutional approval. Each sample was inspected by a pathologist, and normal tissue confirmed. Detrusor smooth muscle was grossly 10 teased from urothelial and serosal layers, snap frozen in liquid nitrogen within 30 minutes of excision, and stored at -70°C for later use. Whole rat bladder was obtained from euthanized male Sprague-Dawley rats (Charles River Laboratories; Wilmington, MA) with institutional animal care committee approval. Rat tissue was harvested within two minutes of death, snap frozen in liquid nitrogen, and 15 stored at -70°C for later use.

**Human detrusor and rat bladder membrane preparation.** Human detrusor and rat whole bladder was minced over dry ice, and suspended in cold lysis buffer (5mM Tris HCl and 5mM EDTA, pH7.4) with protease inhibitors 20 benzamidine (10mg/ml), leupeptin (5mg/ml), and soybean trypsin inhibitor (10mg/ml) (Sigma Chemical Company; St. Louis, MO). A lysate was prepared with a Polytron PT 3000 (Brinkmann; Westbury, NY) at 10,000 rpm for 10 seconds. After pelleting at 40,000 x g for 15 minutes (Sorvall SM24 rotor), membranes were suspended in cold resuspension buffer (150mM NaCl, 50mM 25 TrisHCl, 5mM EDTA, pH7.4) with protease inhibitors, and kept on ice for immediate use (or stored at -70°C for later use). Protein content was determined

using the bicinchoninic assay (BCA) with bovine serum albumin (BSA) standards (Pierce; Rockford, IL).

**Radioligand binding.** All mRNA and protein studies described were 5 performed using detrusor from each patient described above (n=13). In order to conserve sample, and yet fully characterize  $\alpha_1$ ARs in human detrusor, additional full saturation binding isotherms were generated in human detrusor samples from a subset of patients (n=5) using a buffer consisting of 150mM NaCl, 50mM Tris-HCl and 5mM EDTA, pH7.4, with protease inhibitors. Each reaction was 10 performed in triplicate, in a total volume of 0.25 ml, including diluted human detrusor membranes (50 to 100 mg protein) and the  $\alpha_1$ AR antagonist [ $^{125}$ I]HEAT (NEN Research Products-DuPont; Boston, MA) ranging in concentration from 2-900pM; nonspecific binding was measured in the presence of 1mM prazosin (Sigma). The reaction proceeded at 25°C for 45 minutes, and was terminated with 15 five-fold dilution of ice-cold 50mM Tris HCl, pH7.4 buffer, followed by rapid filtration over GF/C filters using a Brandel harvester. Dried filters were then counted in a gamma counter. Specific binding was calculated by subtracting nonspecific binding from total binding. Saturation curves were fit with noniterative regression analysis using InPlot software (GraphPad; San Diego, 20 CA). Total  $\alpha_1$ AR density was then determined in each detrusor sample as described above, using a saturating concentration of [ $^{125}$ I]HEAT (300pM). Results are reported as mean $\pm$ SEM to two significant figures.

To determine Ki values in human detrusor for  $\alpha_1$ AR subtype 25 discriminating ligands, competition binding was performed in triplicate in a total volume of 0.25ml using binding buffer (see saturation binding above). Human

detrusor membranes (50 to 100  $\mu$ g protein) were incubated with a  $K_d$  concentration (120 pM) of the  $\alpha_1$ AR antagonist [ $^{125}$ I]HEAT, and increasing concentrations ( $10^{-12}$  to  $10^{-3}$ M) of the non-radiolabeled  $\alpha_{1d}$ AR-selective ligand BMY7378 (Research Biochemicals International; Natick, MA). Reaction 5 conditions were as described above. Curves were fit with noniterative regression analysis using InPlot software (GraphPad).

**Preparation of RNA.** Total RNA was extracted from human detrusor or rat whole bladder samples using the RNazol method (Tel-Test, Inc.; Friendswood, 10 TX). RNA was quantitated using a spectrophotometer at 260/280 nm, and aliquoted into 20 mg samples for immediate use.

**Human  $\alpha_1$ AR cDNA constructs.** The human  $\alpha_{1a}$ AR probe consists of a 0.326kb (*Pvu*II/*Hind*III) fragment in pGEM-4Z (Promega Corporation; Madison, 15 WI), corresponding to nucleotides 958-1283 of the cloned human  $\alpha_{1a}$ AR cDNA (GenBank #L31774). The human  $\alpha_{1b}$ AR probe consists of a 0.673kb (*Xba*I/*Bam*HI) fragment in pGEM-4Z (Promega), corresponding to nucleotides 94-766 of the cloned human  $\alpha_{1b}$ AR cDNA (GenBank #L31773). The human  $\alpha_{1d}$ AR probe consists of a 0.377kb (*Eco*RI/*Pst*I) fragment, corresponding to 20 nucleotides 520-896 of the cloned human  $\alpha_{1d}$ AR cDNA (GenBank #L31772).

Figure 1 shows the location of each  $\alpha_1$ AR subtype probe within a schematic of the encoded protein. The human cyclophilin probe consists of a 0.103kb (*Kpn*I/*Eco*RI) fragment in pTRI (Ambion, Inc.; Austin, TX), corresponding to nucleotides 38-140 of the cloned human cyclophilin gene (GenBank #X52856).

**Labeling of RNA probes.** Antisense single-stranded radiolabeled RNA probes were generated from linearized  $\alpha_1$ AR cDNA constructs using RNA polymerase T7 ( $\alpha_{1a}$ , cyclophilin) and SP6 ( $\alpha_{1b}$ ,  $\alpha_{1d}$ ) as described in the Promega Protocols and Applications Guide (Promega Corporation; Madison, WI).  $\alpha_{1a}$ AR and  $\alpha_{1d}$ AR cDNA constructs were linearized with *Eco*RI, and the  $\alpha_{1b}$ AR cDNA construct was linearized with *Hind*III.  $^{32}$ P- $\alpha$ UTP (NEN Research Products-DuPont) was incorporated into RNA probes at the time of probe synthesis. All probes were purified on a 5% polyacrylamide gel (300V for 1.5hr); after exposure to film for 3 min, radiolabeled RNA probes were excised from the gel and passively eluted overnight into 400 $\mu$ l of RPA II kit (Ambion) elution buffer at 37°C.

**RNase protection assays.** RNase protection assays were conducted as previously described (Zinn et al, Cell 34:865-879 (1983)) with a few modifications. In brief, total RNA samples (20mg) were dissolved in 20ml of hybridization buffer containing >20-fold excess of radiolabeled probe ( $2 \times 10^5$  cpm/reaction for  $\alpha_{1a}$ ,  $\alpha_{1b}$ ,  $\alpha_{1d}$ , and  $1 \times 10^5$  cpm/reaction for cyclophilin), and incubated overnight at 55°C ( $\alpha_{1a}$ ,  $\alpha_{1b}$ ) and 65°C ( $\alpha_{1d}$ , cyclophilin). To ensure specificity of the synthesized radiolabeled antisense human  $\alpha_1$ AR subtype selective probes, RNase protection assays were performed in tandem with total RNA extracted from rat-1 fibroblast cells stably expressing each cloned human  $\alpha_1$ AR subtype. As a negative control, RNase protection assays for each  $\alpha_1$ AR subtype selective probe were performed in tandem with yeast tRNA samples and other non-hybridizing  $\alpha_1$ AR subtypes. Antisense radiolabeled probe to the highly conserved region of the constitutively expressed human cyclophilin gene was also utilized as a control to ensure identical amounts of total RNA in each assay. The

final gel was exposed to X-Omat AR film (Eastman Kodak Company; Rochester, NY) for 24-72 hours.

**$\alpha_1$ AR mRNA quantitation in human detrusor smooth muscle from**

5 **RNase protection assays.** In order to quantitate relative  $\alpha_1$ AR subtype mRNA, each RNase protection assay final gel was exposed to PhosphorImager plates (Molecular Dynamics; Sunnyvale, CA) for 24 hours. Volume integration of specific protected radiolabeled bands for each mRNA resulting from hybridization products was corrected for background, normalized for cyclophilin signal, and  
10 expressed as arbitrary density units, using ImageQuant gel image-analysis software (Molecular Dynamics).  $\alpha_1$ AR probes contained the following number of UTP sites for  $^{32}$ P- $\alpha$ UTP incorporation:  $\alpha_{1a}$  88,  $\alpha_{1b}$  117,  $\alpha_{1d}$  51. Arbitrary density units were normalized to the lowest  $^{32}$ P- $\alpha$ UTP incorporating probe ( $\alpha_{1d}$ AR) and then expressed as a fraction ( $\pm$ SEM) of total  $\alpha_1$ AR mRNA signal strength.

15

**Polymerase Chain Reaction (PCR).** RT-PCR was used to confirm expression of human detrusor  $\alpha_1$ AR subtypes (to ensure low concentrations of a subtype were not missed in human bladder) and to compare  $\alpha_1$ AR subtype mRNA expression in rat whole bladder with previously published rat data (Walden et al,  
20 J. Urol. 157:1032-1038 (1997), Scofield et al, J. Pharmacol. Exper. Ther. 275:1035-1042 (1995)). Human and rat  $\alpha_1$ AR subtype primers were synthesized at Duke University Medical Center. Reverse transcription of 1mg of DNase-treated human detrusor or rat bladder RNA was performed in triplicate in a 20ml reaction mixture containing 5mM MgCl<sub>2</sub>, 1mM each of dATP, dCTP, dGTP, and  
25 dTTP, 10mM Tris HCl, 50mM KCl, 2ml DEPC treated water, 2.5mM random hexamers, 1 unit of RNase inhibitor, and 2.5 units of MuLV Reverse

Transcriptase (Perkin Elmer; Foster City, CA); simultaneous control samples not treated with reverse transcriptase were used to rule out amplification of genomic DNA. Reverse transcriptase reactions were run for 60 min at 42°C, 5 min at 95°C, and 10 min at 4°C. Each  $\alpha_1$ AR mRNA subtype was amplified by PCR in 5 triplicate in a 100ml reaction containing 50mM KCl, 10mM TrisHCl, pH8.3, 2mM MgCl<sub>2</sub>, 200mM each of dATP, dCTP, dGTP and dTTP, 15pM of sense and antisense primer, 5% DMSO, and 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer). PCR reactions were performed in a DeltaCycler II™ temperature cycler (ERICOMP; San Diego, CA). The following conditions were established 10 for all three rat primer sets: one denaturation cycle for 3 minutes at 95°C, 35 cycles of 1 min at 95°C, 1 min annealing at 58°C, and a 1 min extension at 72°C. The following conditions were established for all three human primer sets: one denaturation cycle for 3 minutes at 95°C, 35 cycles of 1 min at 95°C, 1 min annealing at 60°C for  $\alpha_{1a}$  and  $\alpha_{1b}$ , and 68°C for  $\alpha_{1d}$ , and a 1 min extension at 72°C. 15 A final extension cycle was performed for 10 min at 72°C. Reaction mixtures were then cooled at 4°C. 10ml of each PCR product was separated by gel electrophoresis in 0.8 % agarose. Since PCR experiments were only confirmatory in nature by design, exact quantitation (requiring competitive PCR) was not performed. However, to ensure that any statement regarding relative mRNA 20 levels is appropriate, it is important to note that conditions described above (e.g. different annealing temperatures) were chosen after extensive preliminary analysis with each primer set to ensure optimal amplification conditions with similar primer product efficiency. Equality of reverse transcription efficiency for products was checked using equal concentrations of starting control cDNA; these 25 reactions also served as a positive control for use of correct primer sets. Thirty-five cycles of amplification was chosen since it is at the upper end of the linear

amplification range for all six primer sets ( $\alpha_1$ AR mRNAs are rare at baseline in many human tissues and in our hands 40 cycles of amplification is where the curve becomes non-linear).

5

### EXAMPLE 1

#### **Human Patient Population**

Human detrusor smooth muscle was obtained from male (n=12) and female (n=1) patients. Patient age ranged from 56 to 76 years old (mean = 59.6).  
10 Significant past medical history included tobacco abuse, coronary artery disease, hypertension controlled with  $\alpha_1$ AR or  $\beta$ AR antagonists (n=3), and a history of BOO (n=2) necessitating previous transurethral resection of prostate. Comparison of results from patients with hypertension and/or BOO (n=5) suggests medical history did not affect the results. A larger study would be required to make any  
15 definitive statement in this regard.

### EXAMPLE 2

#### **$\alpha_1$ AR Ligand Saturation Binding**

Pharmacological characteristics of  $\alpha_1$ ARs in human detrusor include a Kd for the radiolabeled  $\alpha_1$ AR antagonist [ $^{125}$ I]HEAT of  $130 \pm 1.9$  pM, similar to that  
20 reported for cells stably expressing the cloned human  $\alpha_{1a}$ AR subtype ((Schwinn et al, J Pharmacol. Exper. Ther. 272:134-142 (1995)). A representative saturation binding isotherm is shown in Figure 2. Total  $\alpha_1$ AR density as measured by saturation binding in human detrusor membrane preparations with the  $\alpha_1$ AR

antagonist [<sup>125</sup>I]HEAT is 6.3±1.0 fmol/mg protein (mean±SEM, range 2.7-9.0, n=13). Although low (with corresponding high non-specific binding of 70-80% as expected),  $\alpha_1$ AR expression is reproducible and consistent within and between patients.

5

### EXAMPLE 3

#### **Identification and Quantification of the $\alpha_1$ AR mRNA Subtypes in Human Bladder Detrusor**

In order to determine which  $\alpha_1$ AR subtypes are present in human detrusor, molecular approaches were chosen due to their sensitivity and specificity. To 10 ensure specificity of the synthesized radiolabeled antisense human  $\alpha_1$ AR subtype selective probes, RNase protection assays were performed simultaneously with total RNA extracted from rat-1 fibroblast cells stably expressing each cloned human  $\alpha_1$ AR subtype. Each  $\alpha_1$ AR subtype specific probe protects a single predominant fragment of predicted size without cross-hybridization (Figure 3, 15 positive control cells); a lack of cross-hybridization between subtypes with each probe (Price et al, Mol. Pharmacol. 46:221-226 (1994)). As was previously demonstrated a further negative control, RNase protection assays for each  $\alpha_1$ AR subtype selective probe were performed in tandem with yeast tRNA samples, where no hybridization is demonstrated (Figure 3, tRNA lane). Human detrusor 20 contains  $\alpha_{1d}$ AR> $\alpha_{1a}$ AR mRNA, but no  $\alpha_{1b}$ AR mRNA in every patient studied (n=13; Figure 3 shows representative results from patients number 1 through 5). This data, when corrected for background, normalized for cyclophilin content, and 25 corrected for probe <sup>32</sup>P- $\alpha$ UTP incorporation, reveals that  $\alpha_{1d}$ AR mRNA constitutes 66±4.8% and  $\alpha_{1a}$ AR mRNA 34±4.8% of the total  $\alpha_1$ AR mRNA in human detrusor.

EXAMPLE 4**Confirmation of  $\alpha_1$ AR Subtype mRNA in Human Detrusor and Comparison with Rat Whole Bladder using RT-PCR**

In order to confirm results from RNase protection assays and to compare

5 with another frequently used animal model (rat),  $\alpha_1$ AR subtype expression was examined using RT-PCR in each patient. Primer nucleotide sequences, melting temperatures ( $T_m$ ), and primer positions relative to the cDNA sequence are shown in Table 1 and Table 2; these primers do not span an intron.

TABLE 1. Oligonucleotide primers used for rat  $\alpha_1$ AR subtype RT-PCR.

Rat $\alpha_1$ AR primers	nucleotide sequences 5'      3'	T <sub>m</sub>	primer position relative to cDNA
$\alpha_{1a}$ AR sense	GTAGCCAAGAGAAAGCCG	62°C	628-647
$\alpha_{1a}$ AR antisense	CAACCCACCACGATGCCAG	66°C	839-820
$\alpha_{1b}$ AR sense	GCTCCTTCTACATCCCGCTCG	68°C	629-649
$\alpha_{1b}$ AR antisense	AGGGGAGCCAACATAAGATGA	62°C	928-908
$\alpha_{1d}$ AR sense	CGTGTGCTCCTTCTACCTACC	66°C	759-779
$\alpha_{1d}$ AR antisense	GCACAGGACGAAGACACCCAC	68°C	1062-1042

TABLE 2. Oligonucleotide primers used for human  $\alpha_1$ AR subtype RT-PCR.

Human $\alpha_1$ AR primers	nucleotide sequences 5'      3'	T <sub>m</sub>	primer position relative to cDNA
$\alpha_{1a}$ AR sense	ATCATCTCCATCGACCGCTACA	66°C	355-376
$\alpha_{1a}$ AR antisense	TCACTTGCTCCGAGTCCGACTT	68°C	697-676
$\alpha_{1b}$ AR sense	GCTCCTTCTACATCCCTCTGG	68°C	629-649
$\alpha_{1b}$ AR antisense	AGGGTAGCCAGCACAAGATGA	67°C	928-908
$\alpha_{1d}$ AR sense	ACCACCGCGAGCCTCGAGGCAGGC	84°C	850-873
$\alpha_{1d}$ AR antisense	GAGCGAGCTGCGGAAGGTGTGGCC	82°C	999-976

Although RNase protection assays are considered the "gold standard" for quantitating mRNA present in a given tissue, this approach is not as sensitive as PCR, therefore very small amounts of mRNA can be missed in a RNase protection assay but demonstrated by PCR. As shown in Figure 4, RT-PCR 5 performed on human detrusor total RNA demonstrates the presence of  $\alpha_{1a}$ AR and  $\alpha_{1d}$ AR mRNA, and lack of  $\alpha_{1b}$ AR mRNA, consistent with data from the RNase protection assays. Of note,  $\alpha_{1d}$ AR mRNA accounts for approximately 60-70% of total  $\alpha_1$ AR mRNA in human detrusor with  $\alpha_{1a}$ AR mRNA accounting for 30-40%, again confirming the RNase protection assay results. Species heterogeneity 10 (human versus rat) of  $\alpha_1$ AR subtype mRNA expression has been previously reported for many tissues (Price et al, Mol. Pharmacol. 46:221-226 (1994), Price et al, Mol. Pharmacol. 45:171-175 (1994)). Indeed, as seen in Figure 4, RT-PCR performed on pooled rat bladder total RNA demonstrates the presence of all three  $\alpha_1$ AR mRNAs in roughly equal concentrations in rat.

15

#### EXAMPLE 5

##### **Determination of $\alpha_1$ AR Subtype Expression at a Protein Level**

Competition analysis was used to determine  $\alpha_1$ AR subtype expression at a protein level in human detrusor. Since molecular studies demonstrate a predominance of  $\alpha_{1d}$ AR mRNA, the  $\alpha_{1d}$ AR-selective compound BMY7378 was 20 used in these studies. As graphically represented in Figure 5, a two-site fit was evident in every patient studied (n=13), with high affinity binding predominating (high  $pK_i = 8.6 \pm 0.2$  [66±3.1% total] vs. low  $pK_i = 4.9 \pm 0.2$  [35±3.1% total]) (Table 3).

**TABLE 3.** Results from competition binding experiments utilizing membranes from rat-1 fibroblasts stably transfected with each  $\alpha_1$ AR subtype (controls) and human detrusor (n=13). Since no  $\alpha_{1b}$ AR was found in human detrusor by RNase protection assays and RT-PCR, one versus two site fit of the data was utilized.

5

BMY7378 (pK <sub>i</sub> )				
	$\alpha_{1a}$ AR	$\alpha_{1b}$ AR	$\alpha_{1d}$ AR	% High
Human detrusor	4.9±0.2	—	8.6±0.2	66±3.1
Control $\alpha_{1a}$	4.8±0.1	—	—	
Control $\alpha_{1b}$	—	5.1±0.3	—	
Control $\alpha_{1d}$	—	—	8.5±0.1	

\* \* \*

All documents cited above are hereby incorporated in their entirety by reference.

10

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

WHAT IS CLAIMED IS:

1. A method of treating irritative symptoms of bladder or lower urinary tract disease comprising administering to a patient in need of such treatment an amount of an  $\alpha_{1d}$ -adrenergic receptor ( $\alpha_{1d}$ AR) antagonist sufficient to effect said treatment.
2. The method according to claim 1 wherein said patient suffers from a disease of the bladder.
3. The method according to claim 1 wherein said patient suffers from a disease of the lower urinary tract.
4. The method according to claim 1 wherein the patient is a female.
5. The method according to claim 4 wherein said  $\alpha_{1d}$ AR antagonist is specific for  $\alpha_{1d}$ AR.
6. The method according to claim 1 wherein the patient is male.
7. The method according to claim 6 wherein said  $\alpha_{1d}$ AR antagonist is an  $\alpha_{1d}$ AR antagonist and an  $\alpha_{1a}$ AR antagonist.
8. The method according to claim 1 wherein said symptoms are selected from the group consisting of frequent urination, urgent urination, nocturia and unstable bladder contractions.

9. A method of treating irritative symptoms of bladder or lower urinary tract disease comprising administering to a patient in need of such treatment an effective amount of a construct comprising an oligonucleotide that inhibits production of  $\alpha_{1d}$ AR or that encodes a molecule that inhibits production of  $\alpha_{1d}$ AR.

10. The method according to claim 9 wherein said construct is an antisense construct.

11. A method of screening a test compound for its ability to bind  $\alpha_{1d}$ AR comprising incubating said test compound with  $\alpha_{1d}$ AR, or portion thereof, and determining the amount of said test compound bound to said  $\alpha_{1d}$ AR, or portion thereof.

12. The method according to claim 11 wherein said portion comprises the transmembrane portion of  $\alpha_{1d}$ AR.

13. The method according to claim 11 wherein said test compound bears a detectable label.

14. The method according to claim 11 wherein said  $\alpha_{1d}$ AR, or portion thereof, is present in a cell membrane.

15. The method according to claim 14 wherein said membrane is the membrane of an intact cell.

16. The method according to claim 15 wherein said cell is a eucaryotic cell.

17. The method according to claim 15 wherein said cell is a cell that has been engineered to express or over-express said  $\alpha_{1d}$ AR, or portion thereof.

18. The method according to claim 11 wherein said test compound is incubated with said  $\alpha_{1d}$ AR, or portion thereof, in the presence of an agent known to bind to  $\alpha_{1d}$ AR, or portion thereof, and the amount of said test compound that binds to said  $\alpha_{1d}$ AR, or portion thereof, is determined indirectly by determining the amount of said agent that binds to said  $\alpha_{1d}$ AR, or portion thereof.

19. The method according to claim 18 wherein said agent bears a detectable label.

20. The method according to claim 18 wherein said agent is BMY7378 or tamsulosin.

21. A compound identified using the method according to claim 11.

22. A composition comprising the compound according to claim 21, and a pharmaceutically acceptable carrier.

23. A kit comprising  $\alpha_{1d}$ AR, or portion thereof, bound to a solid support.

24. A kit comprising  $\alpha_{1d}$ AR, or portion thereof, labeled with a detectable label.
25. A cell engineered to express or over-express  $\alpha_{1d}$ AR, or portion thereof.
26. An antibody specific for  $\alpha_{1d}$ AR.
27. An antisense construct comprising an oligonucleotides, operably linked to a promoter, the transcript of said oligonucleotide being complementary to  $\alpha_{1d}$ AR mRNA.

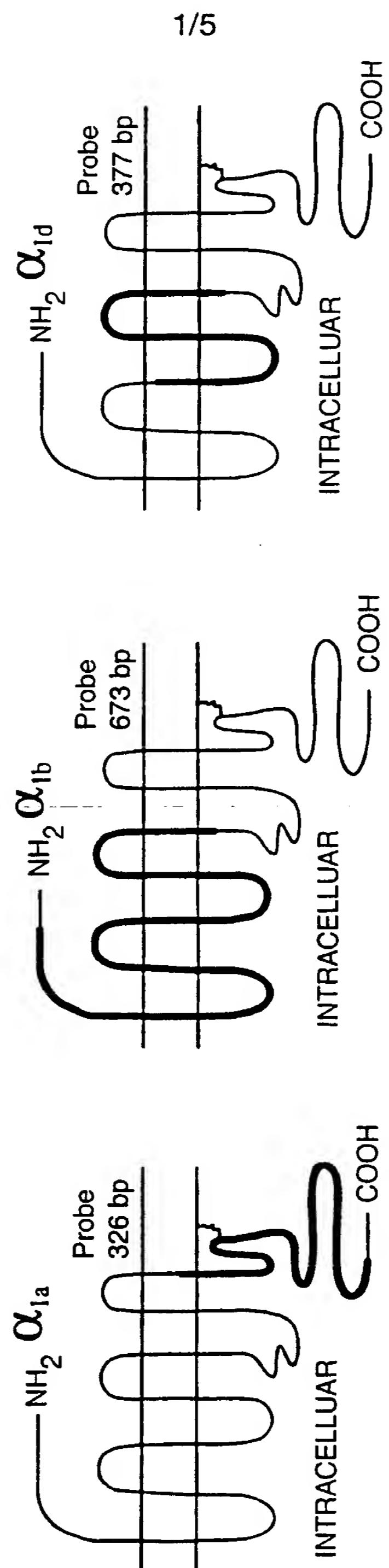


Fig. 1A

Fig. 1B

Fig. 1C

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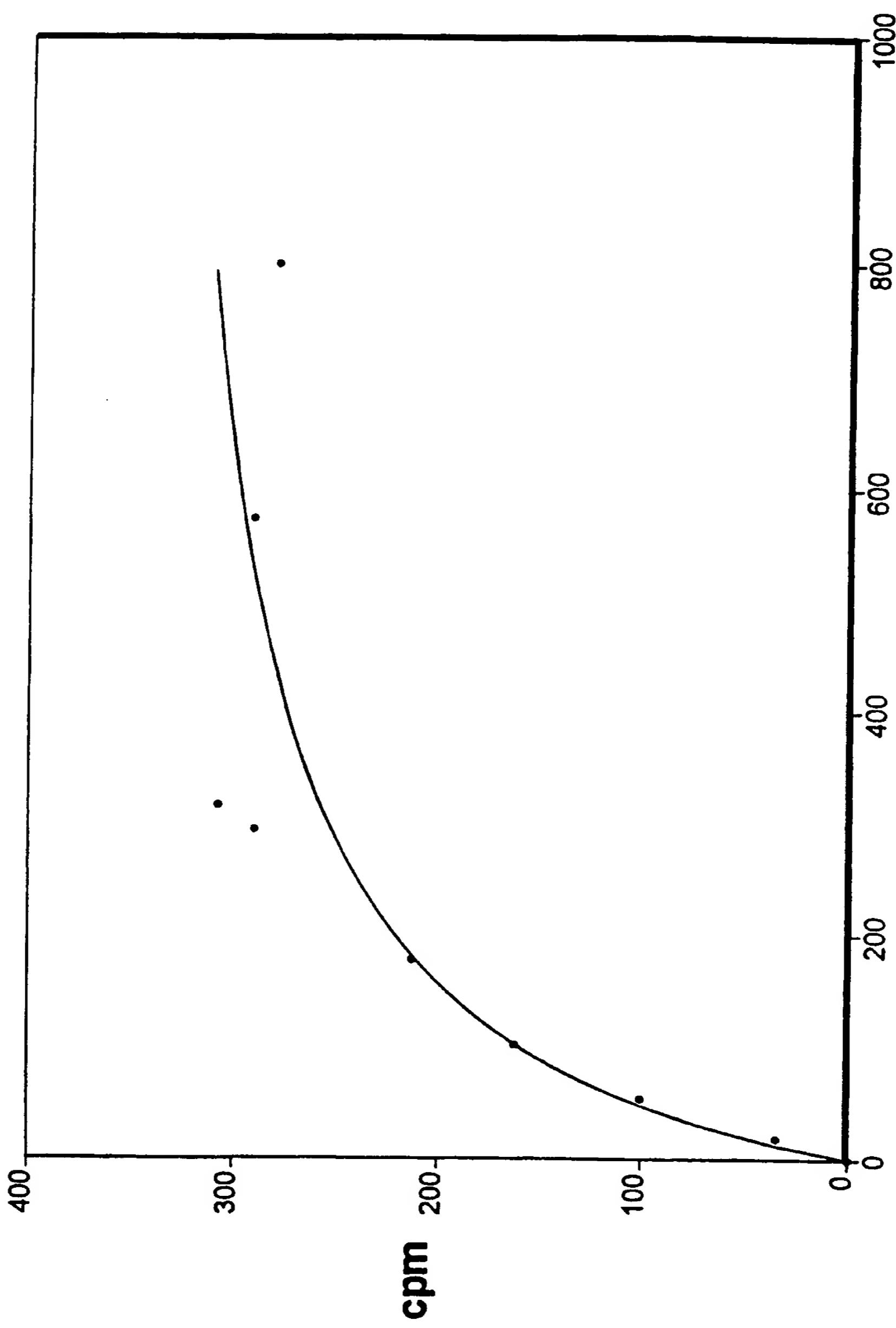


Fig.2

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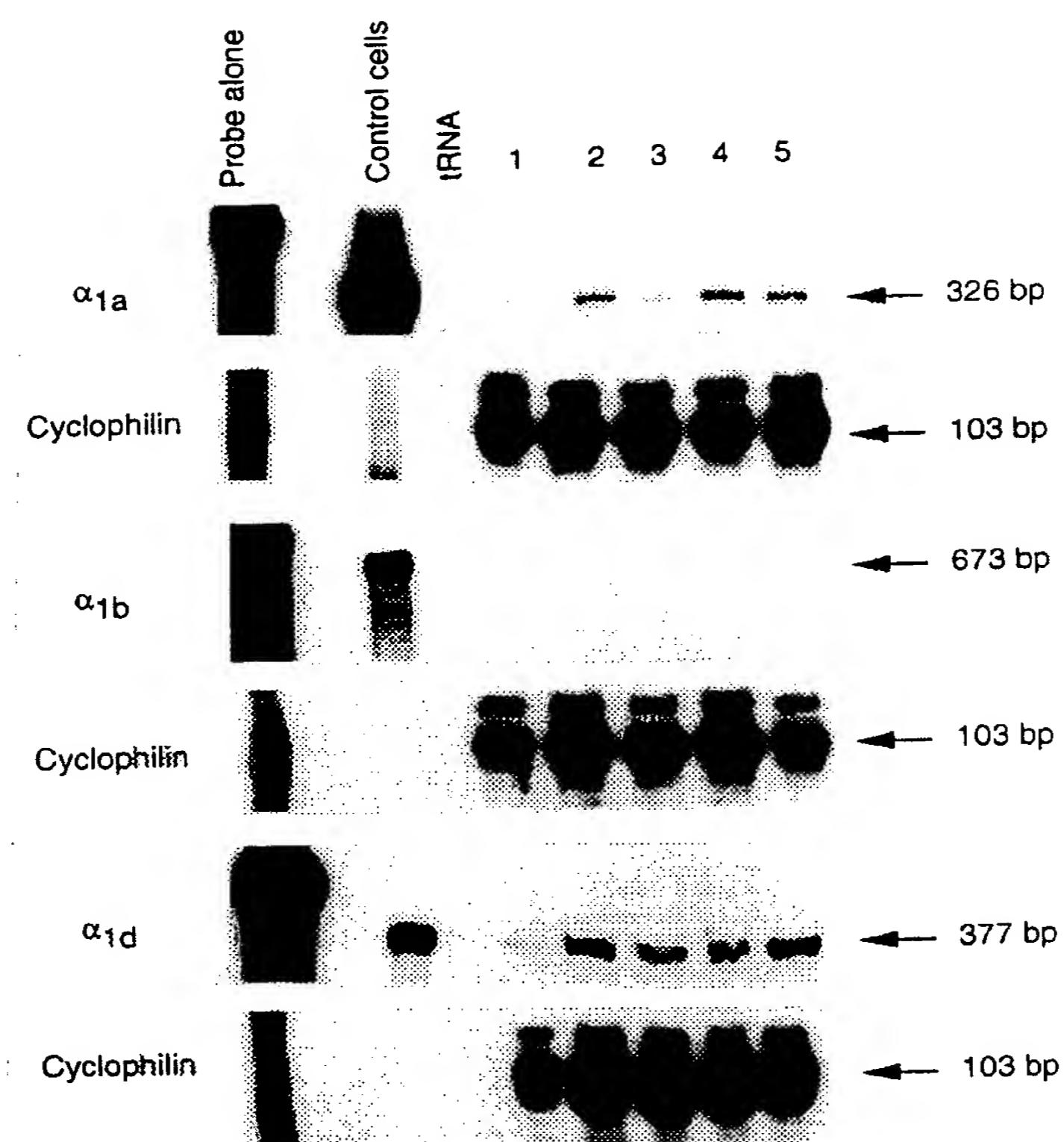


Fig.3

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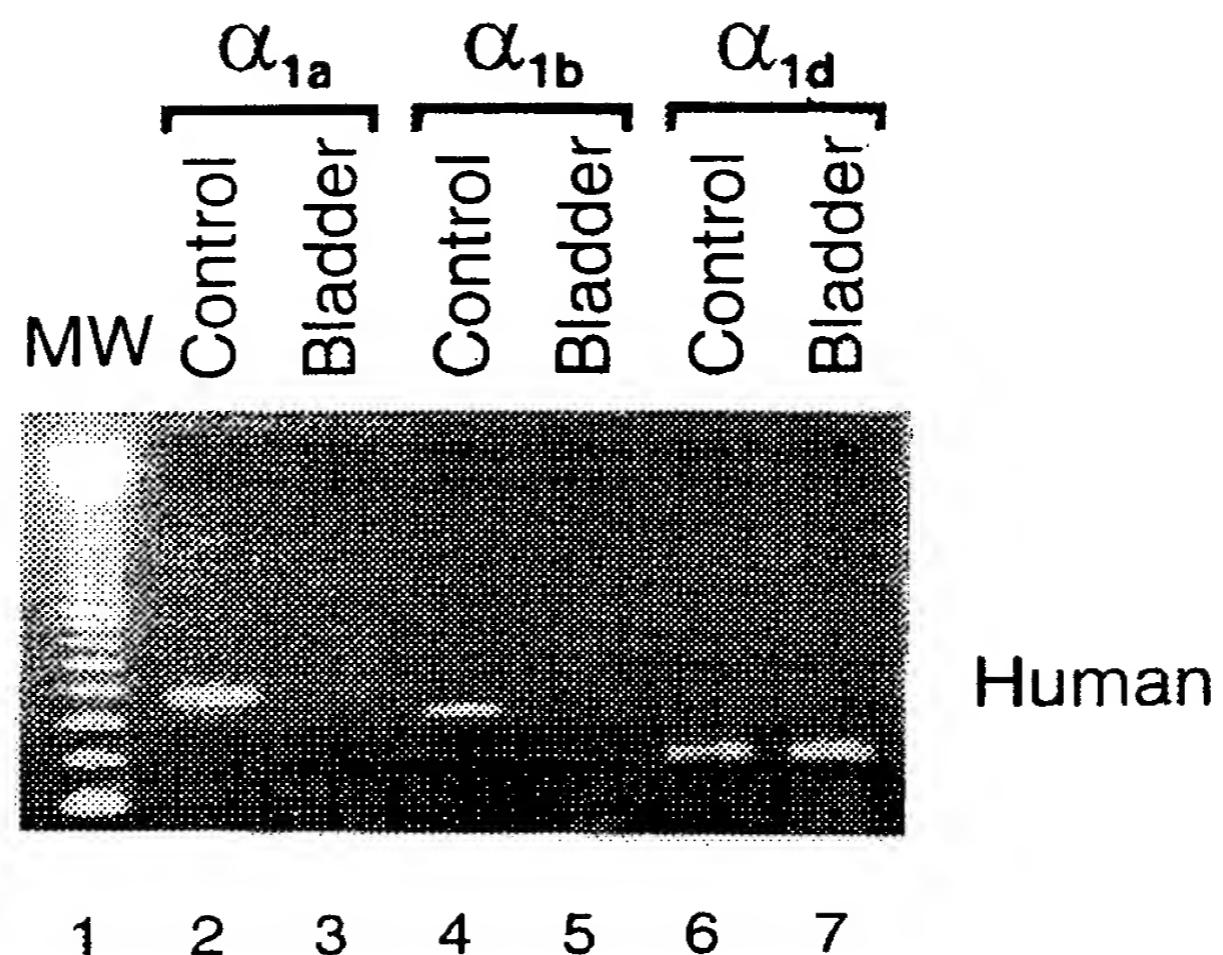


Fig.4A

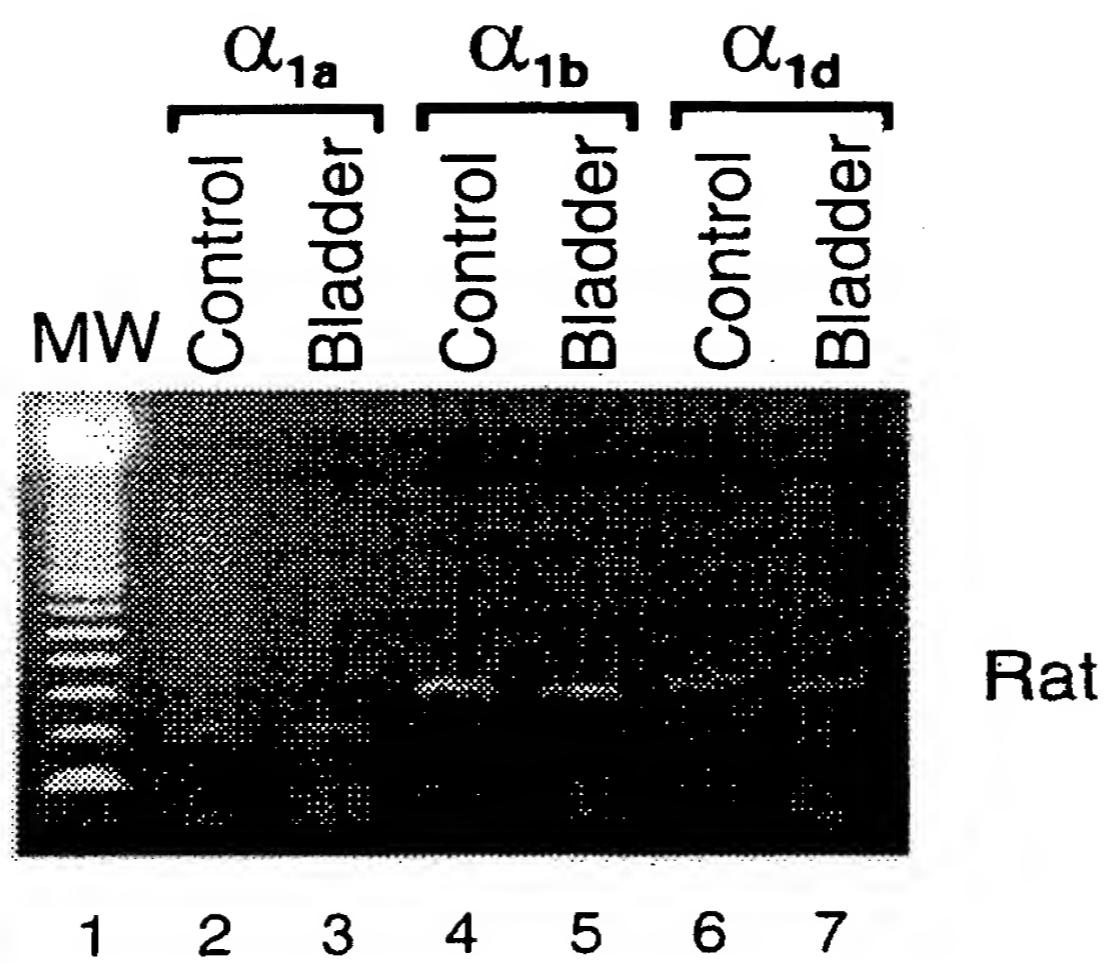


Fig.4B

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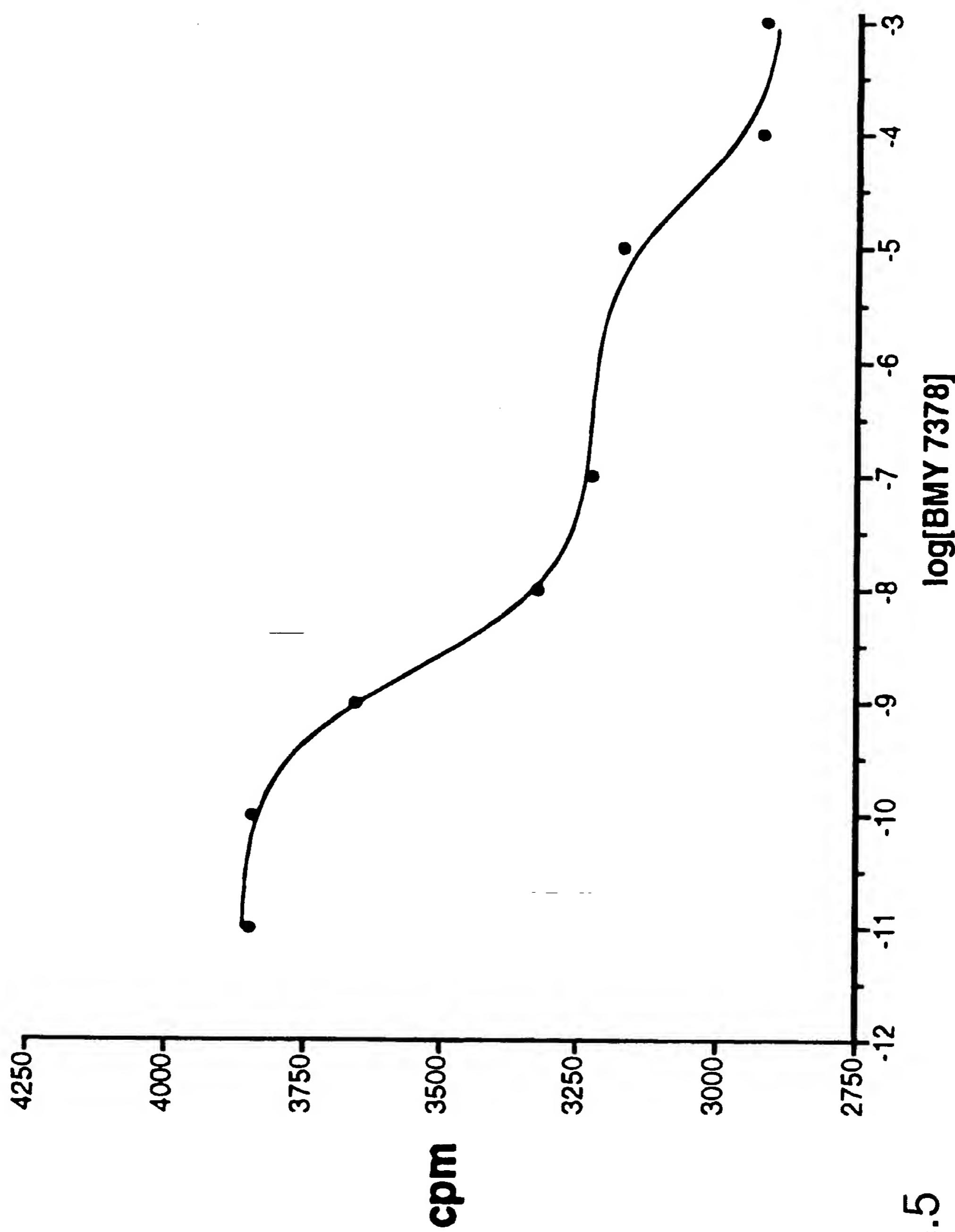


Fig.5

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/09846

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12N 15/85; C07K 14/00, 16/00; A61K 48/00; C12Q 1/68

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.1; 424/130.1; 435/6, 7.1, 7.93, 69.5; 514/44; 536/23.1, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRAHAM et al. Alpha 1-Adrenergic Receptor Subtypes. Circ. Res. 1996, Vol.78, No.5, pages 737-749, see entire document.	1-27
A	DOCHERTY, J.R. Subtypes of functional alpha1- and alpha2-adrenoceptors. Euro. J. Pharmacology. 1998, Vol.361, No.1, pages 1-15, see entire document.	1-27
A,P	Malloy et al. Alphal-Adrenergic Receptor Subtypes In Human Detrusor. J. Urology. September 1998, Vol.160, pages 937-943, see entire document.	1-27

 Further documents are listed in the continuation of Box C.

See patent family annex.

• Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
• "A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
• "E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
• "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
• "O" document referring to an oral disclosure, use, exhibition or other means		
• "P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 JULY 1999

Date of mailing of the international search report

09 SEP 1999

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## INTERNATIONAL SEARCH REPORT

International application No. PCT/US99/09846
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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHARDSON et al. Pharmacology of Tamsulosin: Saturation-Binding Isotherms and Competition Analysis Using Cloned alpha <sub>1</sub> -Adrenergic Receptor Subtypes. <i>The Prostate</i> . 1997, Vol. 33, pages 55-59, see entire document.	1-8, 11-17, 21-25
-		----
Y		18-20, 26, 27
X	MICHEL et al. Alpha-Adrenergic regulation of human renal function. <i>Fundam. Clin. Pharmacol.</i> 1996, Vol. 10, pages 493-503, see entire document.	1-8
-		----
Y		9-27
Y	DOCHERTY et al. The alpha-Adrenoreceptor-Mediated Actions of Chloroethylclonidine. <i>Gen. Pharmacology</i> . 1997, Vol. 28, No. 2, pages 197-201, see entire document.	1-27
X	ANDERSSON et al. Prostatic alpha <sub>1</sub> -Adrenoceptors and Uroselectivity. <i>The Prostate</i> . 1997, Vol. 30, pages 202-215, see entire document.	1-8
-		----
Y		9-27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/09846

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/387.1; 424/130.1; 435/6, 7.1, 7.93, 69.5; 514/44; 536/23.1, 24.5